Therapeutic Alliance: Using N-(2,3,4,5,6-Pentahydroxylhex-1-yl)-N-Dithiocarbamate-*L*-Isoleucine Disodium to Improve the Toxicity and Survival of Cisplatin Receiving Mice

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Abstract: To reduce the toxicity of cisplatin N-(2,3,4,5,6-pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine disodium (GID) based therapeutic alliance is investigated. For the proliferation of $HepG_2$, Hela, MES-SA, HL_{60} and H1299 cells, 27μM of GID based therapeutic alliance gave comparable inhibition to cisplatin alone. For implanted tumor proliferation in mice, 1.667μmol/kg of GID based therapeutic alliance gave higher inhibition than cisplatin alone. For cisplatin receiving mice, this therapeutic alliance effectively reduces the platinum accumulations in the organs but does not affect the platinum level in the tumor tissue. Comparing to cisplatin alone, this therapeutic alliance not only increases urea and fecal platinum levels but also increases urea excretion. All the observations imply that GID based therapeutic alliance is capable of reducing the toxicity and supporting the anti-tumor potency of cisplatin.

Key Words: Cisplatin, N-(2,3,4,5,6-Pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine Disodium, Therapeutic Alliance, Anti-tumor, Toxicity.

1. INTRODUCTION

As one of the most potent chemotherapeutic antitumor drugs cisplatin is not only used against testicular and ovarian cancers but also used against bladder, cervical, head and neck, esophageal, and small cell lung cancer [1]. The wide antitumor actions of cisplatin are believed to be the results of the formation of the bifunctional platinum adducts of cisplatin with DNA and probably a reaction which depends on the initial formation of reactive aquated platinum (II) complexes is involved [2]. In cisplatin therapy however, some tumors have intrinsic resistance to cisplatin and develop acquired resistance [3]. On the other hand, nephrotoxicity has been emphasized as a dose-limiting factor [4,5]. Some processes and substances have been correlated with the nephrotoxicity, such as the oxidative pathways [6], human organic cation transporter 2 in renal proximal tubules [7], and gene expression [8]. The prevention of nephrotoxicity has been considered to be of clinically great importance in the cancer chemotherapy with cisplatin. The toxic effect of cisplatin has greatly hampered its application [9,10], and a wide variety of agents such as selenium, vitamin C, edaravone, cystone, erdosteine and tiopronin were co-administrated to decrease cisplatin-induced nephrotoxicity in the past few decades [11-16]. In fact, the free radical damage mechanism is inherently related with the direct contribution of platinum itself. It is usually considered that in the human body a series of targets other than DNA are able to bind with ciaplatin and lead to multiple effects excluding undesirable effects. For example, among the binding cisplatin only 5% to10% of covalently bound cell-associated cisplatin is found in DNA, whereas 75% to 85% of cisplatin binds to proteins [17,18]. As the first step of the accumulation inside cells, cisplatin could bind to phospholipids and phosphatidylserine of the cell membrane [19]. On the other hand, approaching cytoplasm cisplatin may react with the soft nucleophilic sites of cytoskeletal microfilaments, thiol-containing peptides, proteins and RNA [20]. Same as cisplatin-induced DNA damage of tumor cells, cisplatin-induced DNA or protein damage may directly or indirectly promote normal cells to programmed death [21,22]. With regard to normal tissues and organs, cisplatin-induced DNA and protein damage is a key step to induce cisplatin toxonosis based disorders such as kidney, intestines, stomach, marrow, nerve and ear disorders. As an immediate result a preliminary treatments of cisplatin may cause 33% patients suffer from kidney damage. While continuous treatments of cisplatin the incidence of kidney damage of patients may clamber to 75% [23,24]. This status attracts a lot of interests dealing with platinum itself to lower the nephrotoxicity.

The facts that elevated levels of heavy metals may lead to impairments of the central nervous system such as delayed cognitive development, reduced IQ scores, impaired hearing, and neurobehavioral deficiencies prompt us pay a great deal of interests to develop antagonists of lead and cadmium intoxication. Our previous studies demonstrated that as heavy metal decorporation substances glycosylamines and glycosyldithiocarbamate-*L*-amino acids are highly potency and

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hypotoxicity [25-28]. The similarity of lead, cadmium and platinum intoxication encourages us preparing 20 glycosyldithiocarbamate-*L*-amino acid disodiums (Fig. **1**) as the partners of cisplatin in their therapeutic alliance to improve anticancer therapy.

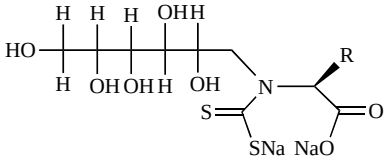

Fig. (1). Formula of glycosyldithiocarbamate-*L*-amino acid disodiums, wherein R represents the side chain of *L*-amino acids, for GID R= $CH(CH_3)CH_2CH_3$.

The preliminary screening explored that *via* therapeutic alliance 20 glycosyldithiocarbamate-*L*-amino acid disodiums were able to reduce the organ platinum levels, toxicity and mortality of cisplatin receiving mice. The detailed *in vitro* and *in vivo* biological activity data of these 20 glycosyldithiocarbamate-*L*-amino acid disodiums from the therapeutic alliance will be reported elsewhere. In the present paper we select GID, one of the most potency compounds, to explore the benefit of this therapeutic alliance.

2. MATERIALS AND METHODS

2.1. Reagents and Instruments in Chemical Synthesis

All the reactions were carried out under nitrogen (1 bar). ^1H (300 and 500 M Hz) and ^{13}C (75 and 125 M Hz) NMR spectra were recorded on Bruker AMX-300 and AMX-500 spectrometers for solution of D_2O, DMSO-d_6, or $CDCl_3$ with tetramethylsilane as internal standard. IR spectra were recorded on a Perkin-Elmer 983 instrument. FAB/MS was determined on VG-ZAB-MS and TOF-MS was recorded on MDS SCIEX QSTAR. Melting points were measured on a XT5 hot stage microscope (Beijing key electro-optic factory). Optical rotations were determined with a Jasco P-1020 Polarimeter at 20ºC. All *L*-amino acids and α-*D*-glucose were purchased from China Biochemical Corp. TLC was made with Qingdao silica gel GF254. Chromatography was performed with Qingdao silica gel H_{60} or Sephadex-LH_{20}. All solvents were distilled and dried before use by reference to literature procedures. Cisplatin (Platinol, crystalline) was purchased from Qilu Pharmaceutical Co. Ltd, China.

2.2. Preparing GID

To the solution of 0.40 g (10 mmol) of NaOH in 3 ml of methanol/water (1:1) 1.31g (10 mmol) of *L*-Ile was added. The mixture was stirred at room temperature for 20 min and *L*-Ile was completely dissolved. Then to the solution, 1.80 g (10 mmol) of α-*D*-glucose was added. The mixture was stirred at room temperature until α-*D*-glucose was completely dissolved. Under the protection of argon gas the solution was stirred at 50-60°C for 5 h and then in situ treated with 1.62 g (30mmol) of sodium borohydrate at room temperature for 96 h. The reaction mixture was cooled to 0°C and adjusted to pH 2.5 by adding concentrated hydrochloric acid. The formed precipitates were removed by filtration and the filtrate was evaporated under vacuum. The residue was diluted with anhydrous ethanol and the formed precipitates were removed by filtration. This procedure was repeated for 5 times and the residue was dissolved in 10 ml of water. The solution was loaded on the column of acidic ion exchange resin and eluted with 3% aqueous solution of N-methylmorpholine to give 1239 mg (42%) of N-(2,3,4,5,6-pentahydroxylhexyl)-*L*-isoleucine as a colorless powder (Mp 228-230°C, ESI (m/e) 296$[M+H]^+$).

The suspension of 295mg (1 mmol) of N-(2,3,4,5,6-pentahydroxylhexyl)-*L*-isoleucine in 5ml of water was stirred at 0ºC for 10 min and then 40 mg (1 mmol) of NaOH was added to form a clean solution. To this solution a solution of 304 mg (4 mmol) of CS_2, 40 mg (1 mmol) of NaOH, 0.4 ml of dioxane and 2 ml of water were added. The reaction mixture was stirred at 0ºC for 5 min and 4 ml of the solution of 202 mg (2 mmol) of triethylamine in 4 ml of tetrahydrofuran was added. The reaction mixture was stirred at 0ºC for 24 h. The reaction mixture was filtered and the filtrate was evaporated under vacuum. The residue was crystallized in aqueous acetone to give 367 mg (88%) of GID as a yellow powder (ESI-MS (m/e) 417$[M+H]^+$). Its HPLC purity was more than 98%.

2.3. *In Vitro* Anti-proliferation Assay of the Therapeutic Alliance

In the evaluation of the anti-proliferation, human carcinoma cell lung carcinoma (H1299), liver carcinoma ($Hep-G_2$), cervical carcinoma (Hela), immature granulocyte leukemia (HL_{60}) and doxorubicin sensitive human sarcoma (MES-SA) cells were cultivated at 37°C in 75 cm^2 flask, to which 12 ml of RPMI1640 (Gibco Laboratories, Santa Clara, CA, USA) containing 10% fetal calf serum (Gibco Laboratories) was added. The cells were grown at a density of 1×10^5 cells/ml and incubated in humidified air with 5% CO_2. The antibiotics were administered in the concentrations of 100U/ml and 100μg/ml for penicillin and streptomycin, respectively.

The proliferations of H1299, HepG2, Hela, HL_{60}, and MES-SA cells were determined using the colorimetric MTT assay as described previously (Mosmann, 1983). Briefly, cells were seeded at a density of 5×10^4 cells/well in a 100μl volume of the medium in 96-well plates (Corning, NY, USA). As the treating group to each well of 9 wells 12.5 μl of cisplatin in NS (final concentration, 333 μM) and 12.5 μl of GID (final concentrations from 27 μM to 16667μM) in NS were added, as the positive control to each well of 9 wells 25 μl of cisplatin in NS (final concentration, 333 μM) was added, as the negative control 25 μl of NS was added. The 96-well plates were incubated for 48 h, centifuged at 2000r/min for 10 min and the supernatant was aspirated. To each well's residue 20 μl of MTT (5 g/l) was added. The 96-well plates were incubated at 37°C for 4 h and the supernatant was aspirated. To each well's residue 100 μl of DMSO was added and then shaken for 8 min. The absorbance was measured at 570 nm (reference at 630 nm) on a 96-well microplate reader (Mode 680, Bio-Rad).

2.4. *In Vivo* Anti-tumor Assay for the Therapeutic Alliance

Male ICR mice (Experimental Animal Center of Capital Medical University) approximately 10–12 week old were maintained in individual stainless steel wire bottom cages

suspended on racks. The mice were kept under carefully controlled conditions of 12-h light/dark cycle, 21°C and 50±20% relative humidity. The mice were acclimated to this environment for 4–7 days prior to the start of the study. S180 was used transplanting the mice to form solid tumors and given by subcutaneous injection. S180 cells for initiation of subcutaneous tumors were obtained from the ascitic form of the tumors in mice, which were serially transplanted once per week. Subcutaneous tumors were implanted by injecting 0.2 ml of 0.9% saline containing 4×10^6 viable tumor cells under the skin on the right oxter. Twenty-four hours after implantation, the mice (10 per group) were randomly divided into experimental groups. The mice of the positive control group were given a daily i.p injection of 16.67μmol/kg of cisplatin (Platinol, Qilu Pharmaceutical Factory, China) in 0.2 ml of 0.9% saline for ten consecutive days. The mice of the negative control group were given a daily i.p injection of 0.2 ml of 0.9% saline for ten consecutive days. The mice of the treatment groups were given a daily i.p injection of 16.67μmol/kg of cisplatin in 0.2 ml of 0.9% saline and 16.67 μmol/kg of GID in 0.2 ml of 0.9% saline for ten consecutive days. On the first day, 2 h after the administration the urine samples of each group were continually collected for 5 h and the fecal samples of each group were continually collected for 24 h. Twenty-four hours after the last administration, all mice were weighed and blood was drawn from the eye orbit. Then the mice were sacrificed by diethyl ether anesthesia and dissected to immediately obtain and weigh the tumor, liver, kidney, brain, spleen, heart and left femur samples. All of the biosamples were digested in $HClO_4$ and HNO_3 (1:3) on a heating block, dried at 80°C, re-dissolved in 1% nitric acid to determine the content of platinum using a Varian Spectr AA-40 atomic absorption spectrometer in the graphite furnace.

The studies with mice described above were performed according to a protocol reviewed and approved by the ethics committee of Capital Medical University. The committee ensures that the welfare of the animals is maintained in accordance to the requirements of the Animal Welfare Act and according to the Guide for Care and Use of Laboratory Animals.

The data obtained from the assays of Items **2.3** to **2.5** were presented as mean values ± SEM and the statistical analysis of the data was carried out using ANOVA test, $P<0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1. Two-step-procedure Preparing GID

The synthetic route of GID included a two-step-procedure. The first step related to the preparation of N-(2,3,4,5,6-pentahydroxylhexyl)-*L*-isoleucine *via* (i) condensing glucose and *L*-isoleucine, (ii) reducing the Schiff base and (iii) acidifying the sodium carboxylates. The obvious features of this simplified one-pot-three-step synthesis are that it can be applied to a variety of natural amino acids, need no tedious separation steps and provided N-(2,3,4,5,6-pentahydroxylhexyl)-*L*-isoleucine in acceptable total yield (42%).

The second step related to the preparation of GID *via* (i) coupling N-(2,3,4,5,6-pentahydroxylhexyl)-*L*-isoleucine and disulfide, and (ii) neutralizing N-(2,3,4,5,6-pentahydroxylhexyl)-N-dithiocarbamate-*L*-isoleucine with sodium hydroxide. As same as the one-pot-three-step procedure mentioned above this one-pot-two-step synthesis have also obvious features such as it can be applied to a variety of N-(2,3,4,5,6-pentahydroxylhexyl)-*L*-amino acids, need no tedious separation steps and provided GID in high total yield (88%).

3.2. Effect of GID on the *In Vitro* Anti-proliferation Activity of Cisplatin

In the *in vitro* assays, the effect of GID as a partner of cisplatin in the therapeutic alliance on the *in vitro* anti-proliferation activity of cicplatin was evaluated using HL_{60}, H1299, $HepG_2$, Hela and MES-SA cells. The growth inhibition values are listed in Table **1**. The data indicate that cisplatin induced growth inhibition to all five cell lines was affected by GID in a dose dependent manner. It was particularly noticed that 27 μM of GID remained cisplatin induced growth inhibition to $HepG_2$, Hela, and MES-SA cells, and enhanced cisplatin induced growth inhibition to HL_{60} and H1299 cells. With the increase of GID's concentration cisplatin induced growth inhibitions to all five cell lines were lowered significantly. In the mentioned MTT assay cisplatin was directly mixed with GID. It is clear that cisplatin is a coordination complex and GID is a chelating agent. Treating cisplatin directly with high concentration of GID may result in decomposition of cisplatin. This should be responsible for higher concentration of GID lowering cisplatin induced growth inhibition to five cell lines.

Table 1. Effect of Therapeutic Alliance of Cp with GID on the Growth of Carcinoma Cells [a]

Cell Lines	% Inhibition of 333 μM Cp with					
	0μM GID	27μM GID	133μM GID	667μM GID	3333μM GID	16667μM GID
HL_{60}	75.05 ±2.94	81.87 ±1.50[b]	77.87 ±1.28	74.99 ±1.97	54.70 ±1.33	15.55 ±6.21
H1299	76.68 ±0.94	82.54 ±0.94[b]	77.81 ±1.92	76.18 ±1.31	53.36 ±1.14	14.34 ±6.29
$HepG_2$	80.68 ±1.72	81.69 ±2.33[c]	69.93 ±1.94	54.72 ±4.22	18.83 ±2.79	12.51 ±5.03
Hela	77.97 ±1.55	78.97 ±1.78[c]	66.96 ±2.05	54.94 ±2.59	18.90 ±4.21	12.89 ±6.89
MES-SA	79.93 ±1.60	79.00 ±2.23[c]	65.00 ±3.25	38.43 ±5.31	21.72 ±4.29	11.68 ±4.35

a) Cp=Cisplatin; GID=N-(2,3,4,5,6-pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine disodium; n=9; b) Compared to Cp alone $p<0.01$, c) Compared to Cp alone $p>0.05$.

3.3. Effect of GID on the *In Vivo* Anti-tumor Activity of Cisplatin

In the *in vivo* assays, the effect of GID as a partner of cisplatin in the therapeutic alliance on the *in vivo* anti-tumor activity of cicplatin was evaluated using S180 mice and the tumor weights are listed in Table **2**. Giving a daily i.p injection of the solution of 16.67 μmol/kg of cisplatin in 0.2 ml of

NS for ten consecutive days the tumor weight was significantly reduced from 1412.20±247.79mg for NS receiving mice to 196.05±42.08mg for cisplatin receiving mice ($p<0.01$). Giving a daily i.p injection of the solution of 16.67μmol/kg of cisplatin in 0.2 ml of NS and the solution of 16.67μmol/kg of GID in 0.2 ml of NS for ten consecutive days the tumor weight was significantly reduced from 1412.20±247.79mg of NS receiving mice to 156.40±40.59 mg of therapeutic alliance mice ($p<0.01$). At the dose of 16.67μmol/kg GID significantly enhance the anti-tumor activity of cisplatin. This enhanced action was accompanied by lowering the tumor weight from 196.05±42.08mg of cisplatin alone receiving mice to 156.40±40.59mg of therapeutic alliance mice, or accompanied by increasing inhibition from 86.48% for cisplatin alone receiving mice to 88.93% for therapeutic alliance mice.

Table 2. Effects of the Therapeutic Alliance on Tumor Weight of S180 Mice[a]

Group	Tumor Weight (mg)	% Inhibition
NS	1412.20±247.79	0
Cp alone	196.05±42.08[b]	86.48
Cp + GID	156.40±40.59[c]	88.93

a) Cp=Cisplatin; GID=N-(2,3,4,5,6-pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine disodium; Tumor weight is expressed by $\bar{X}$ ±SD mg, NS (normal saline) =vehicle, n=10; b) Compared to NS $p<0.01$; c) Compared to NS $p<0.01$ and to Cp alone $p<0.05$.

3.4. *In Vivo* Anti-tumor Action of GID Alone

To evaluate the possibility of GID alone as anti-tumor agent, it was given to S180 mice a daily i.p injection (dose, 84 μmol/kg in 0.2 ml of NS) for ten consecutive days and the tumor weight was recorded. It was found that the tumor weight was 1201.56±238.08 mg (compare to 1412.20±247. 79 mg of NS receiving mice, $p>0.05$). This observation means that GID itself possesses4 no obvious anti-tumor activity.

3.5. Effect of GID on the Tumor Platinum Level of Cisplatin Receiving Mice

For desirable cisplatin therapy a sufficient dose of tumor cisplatin is critical. To examine the effect of the therapeutic alliance on tumor cisplatin the platinum level of S180 mice's tumor was determined. Following the procedure of item 2.4 the mice were treated, the tumors were digested and the platinum contents were determined. After treatment for ten consecutive days the contents of platinum were 5.29±1.62 μg and 4.38±0.79 μg of platinum per g of tumor for cisplatin alone receiving mice and the therapeutic alliance mice, respectively (Table **3**). No significant difference is noticed between the two values ($p>0.05$) and thus the therapeutic alliance did not decrease the tumor platinum level.

3.6. Effect of GID on the Organ Platinum Level of Cisplatin Receiving Mice

The side effects of cisplatin therapy are the general result of platinum accumulation in organs. To examine the effect of therapeutic alliance on platinum accumulation the platinum levels of spleens, kidneys, livers, brains and hearts of S180 mice were determined. Following the procedure of item 2.4 the mice were treated, the spleens, kidneys, livers, brains and hearts were digested, the platinum contents were determined and the data are listed in Table **3**. It was found that in per g of organ the platinum contents of cisplatin alone receiving mice were significantly higher than those of the therapeutic alliance mice, meaning that all the organ platinum levels were significantly decreased by GID.

Table 3. Effects of the Therapeutic Alliance on Organ and Tumor Platinum Levels [a]

Group	Platinum Level in Following Organs		
	Cp Alone	Cp+ GID	NS
Spleen	6.05±1.35	4.12±0.98[b]	0
Kidney	12.41±2.67	4.96±1.36[c]	0
Liver	10.68±1.86	5.96±0.72[c]	0
Brain	0.70±0.22	0.23±0.09[c]	0
Heart	1.58±0.59	0.35±0.07[c]	0
Tumor	5.29±1.62	4.38±0.79[d]	0

a) Cp=Cisplatin; GID=N-(2,3,4,5,6-pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine disodium; Organ platinum is expressed by $\bar{X}$ ±SD μg platinum per g of organ; n=10; b) Compared to Cp alone $p<0.01$, c) Compared to Cp alone $p<0.001$, d) Compared to Cp alone $p>0.05$.

3.7. Effect of GID on Urea, Fecal and Blood Platinum of Cisplatin Receiving Mice

To compare the urea, fecal and blood platinum of cisplatin alone receiving mice and the therapeutic alliance mice the corresponding platinum levels were determined. Following the procedure of item 2.4 the mice were treated, the urea, fecal and blood were digested, the platinum contents were determined and the data are listed in Table **4**. It was found that the platinum contents of the urea and fecal of cisplatin alone receiving mice were significantly higher than those of the therapeutic alliance mice. On the other hand the platinum content of the blood of cisplatin alone receiving mice was significantly lower than that of the therapeutic alliance mice. The observations indicate that *via* the therapeutic alliance the platinum accumulation in the mouse bodies resulted from cisplatin therapy can be effectively avoided *via* excretion of urea and fecal.

3.8. Effect of GID on Urea Quantity of Cisplatin Receiving Mice

To examine the effect of the therapeutic alliance on the urea quantity the urea quantity of cisplatin alone receiving mice and the therapeutic alliance mice was compared. Following the procedure of item 2.4 the mice were treated, the urea was collected and the urea quantity was determined. The data indicate that the urea quantity (1.35±0.35 ml) of NS receiving mice is significantly higher ($n=10$, $p<0.001$) than that (0.58±0.19 ml) of 16.67μmol/kg of cisplatin alone receiving mice. On the other hand however the urea quantities

(2.26±0.21 ml) of the therapeutic alliance mice is significantly higher (n=10, p<0.001) than that of NS and cisplatin alone receiving mice. This observation suggests that comparing to normal mice cisplatin treatment decreases urination and the therapeutic alliance increases urination. This observation also indicates that *via* the therapeutic alliance the platinum accumulation in the mouse bodies resulted from cisplatin therapy can be effectively avoided *via* increasing urination.

Table 4. Effects of the Therapeutic Alliance on Urea, Fecal and Blood Platinum [a]

Group	Urea Platinum	Fecal Platinum	Blood Platinum
NS	0	0	0
Cp alone	26.78±3.26	8.36±2.25	6.19±1.11
Cp + GID	31.24±3.56[b]	10.62±2.26[b]	1.39±0.42[c]

a) Cp=cisplatin; GID=N-(2,3,4,5,6-pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine disodium; The urea and fecal platinum is expressed by $\bar{X}$ ±SDμg platinum per g of urea and fecal; blood platinum is expressed by $\bar{X}$ ±SDμg platinum per ml of blood, n=10; b) Compared to cisplatin alone p<0.05; c) Compared to cisplatin alone, p<0.001.

3.9. Effect of GID on Body and Organ Weights of Cisplatin Receiving Mice

One of visible results of cisplatin toxonosis related organ damage should be tissue necrosis, and total result of organ damage should be body emaciation. To evaluate the effect of the therapeutic alliance on cisplatin toxonosis related organ damage the body weights, absolute organ weights and relative organ weights of S180 mice were measured. Following the procedure of item **2.4** the mice were treated, the weights of the mice body, kidney, brain, spleen, heart, liver and left femur were determined. It was found that the body weight of NS, cisplatin alone and therapeutic alliance treated mice were 30.62±2.22 g, 20.44±3.40 g and 26.22±2.49 g, respectively (n=10). The body weights of cisplatin alone treated mice are significantly lower than that of both NS and therapeutic alliance treated mice (p<0.01). This observation demonstrates that the therapeutic alliance is beneficial to preventing mice from platinum toxonosis related body damage. On the other hand, except absolute spleen weight (165.26± 31.65mg for NS receiving mice, 48.60±11.59mg for cisplatin alone receiving mice and 109.43±33.62mg for the therapeutic alliance mice) the absolute weights of all other organs of the therapeutic alliance mice approach that of NS receiving mice and are significantly higher than that of cisplatin alone receiving mice. Thus decrease in absolute organ weights will be indication of general weakening. This observation also demonstrates that the therapeutic alliance is beneficial to preventing mice from platinum toxonosis related organ damage. The relative weights of organ/body weight are summarized in Table **5**. The data indicate that besides spleen/body weight all the others are at a comparable level. This means that cisplatin induced body emaciation is more serious than induced organ damage. Therefore some other damage should be also responsible for the body emaciation of cisplatin alone treated mice.

Table 5. Effects of the Therapeutic Alliance on Organ/Body Weight [a]

Group	Organ/ Body Weight, %		
	NS	Cp Alone	Cp + GID
Spleen	0.54±0.10	0.24±0.06[b]	0.42±0.13
Left kidney	0.55±0.06	0.64±0.09	0.61±0.07
Liver	7.02±1.43	6.45±0.65	6.76±1.07
Heart	0.40±0.07	0.35±0.09	0.45±0.08
Brain	0.90±0.10	0.81±0.14	0.95±0.13
Left femur	0.22±0.05	0.30±0.06	0.26±0.06

a) NS (normal saline)=vehicle; Cp=cisplatin; GID=N-(2,3,4,5,6-pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine disodium; n=10; b) Compare to NS and therapeutic alliance p<0.01.

3.10. Effect of GID on Survival of Cisplatin Receiving Mice

As a serious result of cisplatin toxonosis related organ damage is death of mice. To evaluate the effect of the therapeutic alliance on cisplatin toxonosis induced death S180 mice were given a daily i.p injection of NS, the solution of 16.67μmol/kg of cisplatin alone in 0.2 ml of NS, or this solution plus the solution of 16.67μmol/kg of GID in 0.2 ml of NS for ten consecutive days. This was repeated for four times and their survival was recorded. From Table **6** it can be noticed that the total survival of both NS receiving mice and the therapeutic alliance mice was 100%. On the other hand however the total survival of cisplatin alone receiving mice was only 40%. This observation demonstrates that the therapeutic alliance is beneficial to increase the survival of cisplatin receiving mice.

Table 6. Effect of the Therapeutic Alliance on Survival [a]

Group	% Survival on		
	8^{th} day	9^{th} day	10^{th} day
NS	100±0	100±0	100±0
Cp alone	80±7	50±7	40±10
Cp + GID	100±0[b]	100±0[b]	100±0[b]

a) NS (normal saline)=vehicle; Cp=cisplatin; GID=N-(2,3,4,5,6-pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine disodium; Experimental number=4; b) Compared to NS p>0.05 and to Cp alone p<0.001.

4. CONCLUSION

As a dose-limiting factor cisplatin therapy-induced nephrotoxicity attracts a lot of interests and the reduction of the nephrotoxicity has been considered to be of clinically great importance. Based on a chelating mechanism this work provides a therapeutic alliance of GID. Using the suitable assays we found that GID based therapeutic alliance not only reduced the toxic effects but also supports the anti-tumor po-

tency of cisplatin. Comparing to cisplatin alone, GID based therapeutic alliance greatly increased the urea quantity and urea platinum level of the treated mice. These may be considered as the path eliminating the platinum accumulation and reducing the organ damage of the mice receiving the therapeutic alliance. The facts that tumor platinum level of the mice receiving cisplatin alone equals to that of the mice receiving therapeutic alliance provide a sound basis for the latter. However, the reason of the enhanced antitumor activities of cisplatin by the therapeutic alliance remains to be elucidated.

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ABBREVIATIONS

GID = N-(2,3,4,5,6-Pentahydroxylhex-1-yl)-N-dithiocarbamate-*L*-isoleucine Disodium

Cp = Cisplatin

TLC = Thin Layer Chromatography

MTT = 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide

DMSO = Dimethyl sulfoxide

ESI-MS = Electrospray Ionization Mass

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